PATENTS
Attorney Docket No. 7385

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Patent Application of:
Edwin F. Ullman, *et al.*

Serial No.: 09/732,047

Confirmation No.: 9672

Filed: December 7, 2000

Title: Amplified Signal in Binding Assays

Board of Patent Appeals and Interferences
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

APPELLANT'S BRIEF ON APPEAL

This is an appeal from the Final Rejection in the Office Action dated February 6, 2007 (the "Final Rejection"), by the United States Patent and Trademark Office (the "Office") in the above-identified patent application. A Notice of Appeal was mailed on May 3, 2007.

Jurisdiction over this appeal resides in the Board of Patent Appeals and Interferences under 35 U.S.C. §134.

An oral hearing was not requested.

REAL PARTY IN INTEREST

The real party in interest is Dade Behring Marburg GmbH.

RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences.

STATUS OF CLAIMS

The claims for consideration on appeal in the present application are claims 44-46, which are all of the claims remaining in the present application since Claims 1-43 were previously canceled. Claims 44-46 stand rejected.

STATUS OF THE AMENDMENTS

An Amendment under 37 C.F.R. 1.111 (the Amendment) was filed prior to the final rejection; no amendments were made after the Final Rejection. All amendments to the claims made prior to the Final Rejection have been entered and the claims set forth in the Claims Appendix represent the current state of the claims and include all entered amendments.

SUMMARY OF CLAIMED SUBJECT MATTER

Claim 44 is directed to a method for determining the presence or concentration of an analyte in a medium (p 1, ln 7-8, where p = page(s) and ln = line(s) of the specification). A reaction mixture is provided, which comprises in combination (i) a medium suspected of containing an analyte (p 57, ln 7-9); (ii) a first specific binding pair member bound to a water-insoluble solid support (p 57, ln 3-5, and p 23, ln 18-19, and p 24, ln 24; (iii) a second specific binding pair member bound to a sensitizer (p 57, ln 5), said sensitizer capable in its excited state of generating a reactive oxygen species (p 19, ln 19-21, p 116, ln 7-8), wherein the proximity of the first specific binding pair member with the second specific binding pair member is modulated

by the presence of the analyte (p 52, ln 13-16, and p 116, ln 8-10); and (iv) digoxigenin-linked biotin linked to the solid support through a reactive oxygen cleavable linker (p 95, ln 18-20). The reaction mixture is incubated (p 36, ln 14-15). The sensitizer is excited and the excitation of the sensitizer causes the formation of reactive oxygen, which cleaves the cleavable linker and releases digoxigenin-linked biotin from the solid support (p 57, ln 12-14, and p 95, ln 18-20). The released digoxigenin-linked biotin is detected and the amount of the released digoxigenin-linked biotin is related to the amount of analyte in the medium (p 95, ln 21-30).

GROUNDS OF OBJECTION AND REJECTION TO BE REVIEWED ON APPEAL

The following grounds of objection and rejection are presented for review on appeal:

The objection to the specification at page 5, lines 1-14.

The objection to the specification at page 9, lines 5-22.

The rejection of claims 44-46 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The rejection of claims 44-46 under 35 U.S.C. 103(a) as being unpatentable over Bronstein, *et al.* (U.S. Patent No. 6,243,980) (Bronstein) in view of Pease, *et al.* (U.S. Patent No. 5,709,994).

ARGUMENT

The objection to the specification at page 5, lines 1-14.

The Office objected to the amendment to the specification at page 5, lines 1-14, filed December 2, 2005, because, contends the Office, the mechanism by which "release of the substrate with formation of a first binding site may be accompanied by unmasking of at least some of a second binding site" is mechanistically unclear. This objection is basically an objection to the specification based on the description requirement of 35 U.S.C. 1.112, first paragraph, which states that the specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact

terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same.

As can be seen from the file history of this application, the paragraph on page 5, lines 1-14, was amended in the Amendment to refer to "binding sites" to make the language of the original paragraph consistent.

Appellant submits that the present specification does contain a written description concerning the statement that "release of the substrate with formation of a first binding site may be accompanied by unmasking of at least some of a second binding site" where the written description enables any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same.

Masking is described in the specification as a situation whereby a functional group is unable to bind to its specific binding reagent. Such masking can arise simply by virtue of the substrate being bound to a surface. For instance, as set forth in the specification, the substrate may be bound within pores of the support or surface, i.e., an agarose gel, where the pores are too small to accommodate the specific binding reagent. Alternatively, numerous substrate molecules bound to a relatively smooth surface will be unavailable for binding to a specific binding reagent provided that the specific binding reagent is sufficiently bulky as, for example, when it is attached to latex particles.

Particular examples of unmasking are set forth in the specification. For example, the specification discusses a method for the selective protection or masking of biotin and analogues thereof at the ureido nitrogen using a singlet oxygen cleavable group. The method employs a copper catalyzed coupling reaction to couple the ureido nitrogen of biotin with a variety of unsaturated singlet oxygen sensitive compounds such as oxazole and anthracene halides, vinyl halides, and aryl halides. Deprotection or demasking of the biotin is accomplished in the presence of singlet oxygen, which cleaves off the masking group. The cleavable group may function as a protective mask to shield biotin in the presence of proteins such as avidin and streptavidin, which strongly bind to biotin. Alternatively, the cleavable group may function simultaneously as a linker to attach biotin to a molecule, support or surface and as a protective mask to shield the biotin in the presence of binding proteins. Singlet oxygen cleavage of the cleavable group simultaneously frees the biotin from the support or surface and unmasks the biotin, allowing the unmasked biotin to bind to an appropriate protein as desired. In this example,

release of the substrate occurs with formation of the first functional group or binding site that is accompanied by unmasking of at least some of the second functional group or binding site.

The Office Action contends that none of the examples from the specification, which Appellant had set forth, are analogous to a mechanism involving both: (1) formation of a first binding site; and (2) unmasking of a second binding site.

With regard to what the Office has characterized as items (1)-(3) (p 7, third full paragraph of the Final Rejection), Appellant's previous arguments included a general discussion of masking. Item (4), as characterized by the Office, appears to refer to Appellant's discussion, both above and in the Amendment, regarding a method for the selective protection or masking of biotin and analogues thereof at the ureido nitrogen using a singlet oxygen cleavable group. Appellant explained that release of the substrate biotin occurs with formation of the first functional group or binding site, namely, the ureido nitrogen, and is accompanied by unmasking of at least some of the second functional group or binding site, namely, the biotin molecule, which is free to bind to a complementary agent. The Office (Final Rejection, p 7, last paragraph) asserts that the present specification has no support for either assignment of "binding site" set forth in item (4).

Appellant submits that the present specification indicates that release of the substrate with formation of the first functional group or binding site may be accompanied by unmasking of at least some of the second functional group or binding site. The specification indicates that singlet oxygen cleavage of the cleavable group simultaneously frees the biotin from the support or surface and unmasks the biotin, allowing the unmasked biotin to bind to an appropriate protein as desired. The free amino group (i.e., ureido nitrogen) on the biotin represents the formation of the first binding site or functional group. The specification (p 5, ln 24-26) indicates that an amine group can be a chemical specific binding reagent. Furthermore, functional groups are indicated in the specification as including amino groups (p 24, ln 9-10). The free ureido nitrogen is, therefore, a functional group or binding site that is formed during the aforementioned cleavage reaction and the unmasked biotin represents the second binding site. The ureido nitrogen of the biotin is free to react with a corresponding chemical specific binding reagent (e.g., a reagent having an electrophilic group (p 5, ln 24-26)) and the unmasked biotin is free to bind to a complementary binding agent or protein (p 6, ln 29, to p 7, ln 1).

Another example is discussed in the specification as a fourth embodiment (for example, paragraph bridging pages 5 and 6). A substrate attached to a support or surface via an oxidant cleavable linker reacts with an oxidant to produce a product having a chemically reactive group, usually an electrophilic group. This chemically reactive group is designed to react with a chemical-specific binding reagent, which can be a nucleophile such as an amine or sulfhydryl. The product therefore becomes covalently bound to the specific binding reagent. The product also contains a hapten or ligand that was originally present in the substrate or unmasked as a result of the oxidation or subsequent reaction. For example, oxidation of a substrate that contains one haptenic group linked to a support through an oxidizable linker can yield an active ester as the chemically reactive group. The specific binding reagent could then be an amine, which reacts with the ester. If the amine is attached to a label, reaction with the oxidation product not only releases the product from the support or polymer but also binds the product to the label. If the amine is not attached to a label, it can react with the product to produce a new group, which can serve as a ligand. A labeled receptor for the ligand can then be used in the subsequent detection step.

The objection to the specification at page 9, lines 5-22

The Office objected to the amendment filed March 31, 2006, because, contends the Office, the following sentence is indefinite: "When peroxide or singlet oxygen is generated, an oxidant cleavable linker is cleaved, releasing multiple products." The Office asserts that it is not clear how cleaving a linker results in release of "multiple products."

First, the amendment referred to by the Office was made in an amendment mailed on November 17, 2006. Second, the context of the paragraph in which the above sentence appears makes it clear to one of ordinary skill in the art how cleaving a linker results in release of multiple products. The language immediately preceding the above sentence states that the "method of the invention entails a first step of forming a sandwich of a first receptor bound to the sensitizer particle, an analyte or target, and a second receptor associated with multiple copies of a substrate. The substrate is attached via oxidant cleavable linker to a support or surface such as a particle to form what is referred herein as an acceptor particle. The analyte binds to the first and second receptor, drawing the catalyst and substrate in close proximity" (underlining added). Therefore, each second receptor molecule has multiple copies of a substrate attached. When

peroxide or singlet oxygen is generated, the oxidant cleavable linker is cleaved releasing multiple products, which are then detected. As can be seen, each cleavage of the cleavable linker results in release of a product and, since multiple copies of the substrate are attached, multiple products are released. As indicated in the specification, the product is distinguished from the substrate by the creation of at least one functional group in the product that was not present in the substrate (p 37, ln 10-12).

The rejection of claims 44-46 under 35 U.S.C. 112, second paragraph

Claims 44-46 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Office asserts that the term "water-insoluble" in claim 44 is indefinite. The Office further asserts that the identity of one or more standards or conditions for ascertaining solubility is not clear and appears omitted from the present specification and that whether claim 44 requires "water" is not clear.

The definiteness of the language employed must be analyzed not in a vacuum, but in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing ordinary level of skill in the pertinent art. *In re Angstadt*, 537 F.2d 498, 190 USPQ 214 (C.C.P.A. 1976). The amount of precision required is relative. If the claims, read in the light of the specification, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and, if the language is as precise as the subject matter permits, the courts can demand no more. *North Am. Vaccine, Inc. v. American Cyanamid Co.*, 7 F.3d 1571, 28 USPQ 2d 1333 (Fed. Cir. 1993).

Appellant submits that the phrase “water insoluble” is not indefinite to one of ordinary skill in the art. This is supported by the fact that a search for patents and published patent applications using the Office’s website showed that 48,201 issued patents and 21,746 published patent applications contained one or more iterations of the phrase “water insoluble.” While, for the sake of time, only a limited number of such patents and patent applications were reviewed, none contained a discussion or definition of the phrase “water insoluble.” One such patent is Pease, *et al.* (U.S. Patent No. 5,709,994), which was applied by the Office in the rejection under 35 U.S.C. 103(a) discussed in detail below. Pease uses the phrase “water insoluble” but does not provide a discussion or definition of the phrase. In view of the above, the phrase “water

insoluble" appears to be so well understood by those skilled in the art that neither the inventors in over 65,000 patents and published patent applications, nor the Office for that matter, seems to require any definition of the phrase or any standards of conditions by which to measure water insolubility. Therefore, the phrase is not indefinite.

The rejection of claims 44-46 under 35 U.S.C. 103(a) as being unpatentable over Bronstein in view of Pease

Claims 44-46 were rejected (and the rejection made final) under paragraph (a) of 35 U.S.C. §103 as being unpatentable over Bronstein in view of Pease. With regard to this rejection, Appellant submits that claims 44-45 on the one hand and claim 46 on the other are separately patentable over Bronstein in view of Pease. The rejection of claims 44-45 as unpatentable over Bronstein in view of Pease is discussed in the following section of the Brief on Appeal. The rejection of claim 44 as unpatentable over Bronstein in view of Pease is discussed in a subsequent section of the Brief on Appeal.

The final rejection of claims 44-45 under 35 U.S.C. 103(a) as being unpatentable over Bronstein in view of Pease

Appellant submits that, even if for the sake of argument one skilled in the art might combine the teachings of Bronstein and Pease, one still would not be in possession of all of the elements of the presently claimed methods. According to M.P.E.P. 2143.03, all claim limitations must be taught or suggested by the prior art in order to establish *prima facie* obviousness (citing *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974)). "All words in a claim must be considered in judging the patentability of that claim against the prior art." *In re Wilson*, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970).

Appellant's argument may be summarized as follows: The combined teaching of Bronstein and Pease is deficient in not disclosing or suggesting at least the following limitations of claim 44: (1) a second specific binding pair member bound to a sensitizer where the sensitizer is capable in its excited state of generating a reactive oxygen species, wherein the proximity of first specific binding pair member with the second specific binding pair member is modulated by the presence of the analyte; (2) digoxigenin-linked biotin linked to the solid support through a reactive oxygen cleavable linker; (3) exciting the sensitizer where the excitation of the sensitizer

causes the formation of reactive oxygen, which cleaves the cleavable linker and releases digoxigenin-linked biotin from the solid support and detecting the released digoxigenin-linked biotin, the amount thereof being related to the amount of analyte in the medium.

The teaching of Bronstein and Pease

Bronstein discloses two distinct assays, namely, a heterogeneous assay and a homogeneous assay, each involving distinct reagents for each type of assay. As discussed by the patentee, a heterogeneous assay is described in the specification and illustrated in Fig. 2A and 2B. At col. 5, line 42, *et seq.*, the patentee indicates that a peptide substrate is synthesized which contains the appropriate cleavage site for the target protease. This peptide is labeled with one member of a first ligand binding pair, such as biotin, on one end and a member of a second ligand binding pair, such as fluorescein, at the other end. This peptide is then incubated with the protease and a compound of interest to be screened for inhibitory activity, in a well or other solid phase coated with the second binding ligand of the first ligand binding pair, such as avidin or streptavidin. In this way, the biotin binds to the streptavidin and the fluorescein-labeled peptide becomes bound to the well unless the peptide was cleaved by the protease in which case only the biotin with a peptide fragment binds to the streptavidin in the well. After incubation, the wells are washed and incubated with the second binding member of the second binding ligand pair conjugated with an enzyme, which is an effective trigger for a 1,2-dioxetane such as alkaline phosphatase. The wells are then washed and incubated with a 1,2-dioxetane substrate such as chlorine substituted phosphate dioxetane (CSPD) (see Fig. 3) and the signal is measured. Higher signals are detected in the presence of an inhibitor. This is so because the inhibitor prevents the protease from cleaving the peptide, and the fluorescein-peptide-biotin moiety bound to the streptavidin attached to the well remains intact. Therefore, the fluorescein is available for binding to the binding member for the fluorescein (second binding member for the first binding member of the second ligand binding pair), which is conjugated to the alkaline phosphatase. Thus, the alkaline phosphatase reagent remains in the well after washing and is available to act on the CSPD substrate resulting in the release of a chemiluminescent species. If the test compound is not a protease inhibitor, the protease cleaves the peptide and there is no fluorescein-peptide-biotin moiety remaining in the well after washing.

In a homogenous assay of Bronstein, the same substrate bears at one end a fluorescent

energy accepting moiety and at the other end a 1,2-dioxetane or precursor (see col. 7, ln 38-63, and Figs. 14A and 14B). If the substrate is cleaved by the protease, the dioxetane and the fluorescent moiety are not in close physical relationship, and no energy transfer occurs when the dioxetane is caused to decompose. If cleavage has not occurred, which indicates inhibition, the dioxetane is caused to decompose and energy is transferred to the fluorescing entity, which releases light of a wavelength recognizably distinct from that of the dioxetane. The peptide is sufficiently short (no more than about 10 amino acid residues) such that the dioxetane is in close physical association with the fluorescent label. The wavelength of the fluorescent emitter is characteristically shifted markedly from that of the dioxetane, allowing easy discrimination in a homogenous assay.

Pease discusses photoactivatable chemiluminescent matrices and indicates that a solid matrix is a support or surface comprised of a porous or non-porous water insoluble material.

(1) Limitation of claim 44 that recites a second specific binding pair member bound to a sensitizer where the sensitizer is capable in its excited state of generating a reactive oxygen species, wherein the proximity of first specific binding pair member with the second specific binding pair member is modulated by the presence of the analyte

The Office asserts that the alkaline phosphatase of Fig. 3 of Bronstein is the first specific binding pair member, which, asserts the Office, is bound to a support and that the adamantyl moiety is the support. The Office identifies the second specific binding pair member as the $OPO_3^=$ in Fig. 3 bound to a sensitizer referring to a 1,2-dioxetane moiety precursor capable in its excited state of generating a reactive oxygen species, referring to photooxygenation of a dioxetane (col. 5, lines 35-37).

This contention in by the Office is not persuasive for a number of reasons. First, the disclosure in the reference concerning photooxygenation (col. 5, lines 36-37, which is part of a paragraph of Bronstein that discusses Fig. 14) relates to the preparation of the CSPD reagent wherein oxygen is added to the double bond of the precursor to form the 1,2-dioxetane moiety. This disclosure of the reference relates to the reagents of the homogeneous assay of Bronstein whereas Fig. 3 of Bronstein and the corresponding discussion relates to reagents of the heterogeneous assay of Bronstein (see col. 5, ln 64, to col 6, ln 4). The homogeneous assay and the heterogeneous assay of the reference are two distinct assay approaches and utilize different

reagents for each.

Neither disclosure of Bronstein referred to by the Office relates to the present claim limitation. The cleavage process disclosed in Fig. 3 has nothing to do with the claimed sensitizer capable in its excited state of generating a reactive oxygen species where the sensitizer is bound to a second member of a specific binding pair. As is readily seen, the CSPD is a formed reagent that is used in the protease inhibitor assay. It is not a sensitizer reagent as presently claimed and does not generate a reactive oxygen species in the Bronstein assay, especially a reactive oxygen species that cleaves an oxygen-cleavable linker. Cleavages that occur in the heterogeneous assay of Bronstein are cleavage of the synthetic peptide reagent (col. 5, ln 43-44) and cleavage of the 1,2-dioxetane (col. 5, ln 66-67). Cleavage of the synthetic peptide reagent results from the protease that is present during the initial part of the disclosed assay (col. 5, ln 48-49). Cleavage of the 1,2-dioxetane results from the action of the alkaline phosphatase on the $OPO_3^=$ of the CSPD reagent (Fig. 3). Neither of these cleavages involves a reactive oxygen species generated by a sensitizer reagent or the cleavage of an oxygen-cleavable linker.

The Office asserts that one skilled in the art would recognize that the adamantyl moiety of the CSPD reagent of Bronstein could be a water insoluble solid support. It would have been obvious for a person of ordinary skill, contends the Office, to perform the analyte determination of Bronstein using "water-insoluble" solid supports because Pease discovered "water-insoluble" solid supports that allow separation of bound binding partners from unbound binding partners (referring to col. 10, lines 8-12, of Pease). Furthermore, continues the Office, Pease discovered "water-insoluble" solid supports with "delayed luminescence" lifetimes, which can be modulated as a function of structure and/or composition (referring to col. 8, lines 1-9).

As can be seen from Fig. 3 of Bronstein, cleavage of the 1,2-dioxetane results in release of a chemiluminescent moiety, which is detected. Substituting a water insoluble support for the adamantyl moiety provides no apparent advantages to this reaction and detection. The advantages recited by the Office appear to be irrelevant to the chemistry of Bronstein, whose method of Fig. 3 relates to a heterogeneous assay that already utilizes a solid phase (coated plate, see Figs 2A and 2B). The CSPD reagent is added to the plate after the protease inhibition reaction has been allowed to proceed and the plate has been washed. Having the CSPD reagent in a solid phase would hinder the ability of the CSPD reagent to diffuse to the area of the fluorescein moiety on the peptide. Such diffusion is important for the success of the detection

step of Bronstein's heterogeneous assay, which requires energy transfer between the CSPD reagent and the fluorescein moiety. In any event, as discussed above, even if for the sake of argument the proposed substitution were made, one skilled in the art would still not be in possession of the claim limitation of a second specific binding pair member bound to a sensitizer where the sensitizer is capable in its excited state of generating a reactive oxygen species.

With regard to the homogeneous assay format of Bronstein, substitution of a solid phase for the adamantyl group of the reagent depicted in Fig. 14 and discussed at col. 7, ln 38-63, would change the entire character of the assay from homogeneous to heterogeneous. Furthermore, as discussed above, even if for the sake of argument the proposed substitution were made, one skilled in the art would still not be in possession of the claim limitation of a second specific binding pair member bound to a sensitizer where the sensitizer is capable in its excited state of generating a reactive oxygen species.

(2) Limitation of claim 44 that recites digoxigenin-linked biotin linked to the solid support through a reactive oxygen cleavable linker

As discussed above, Bronstein does not disclose or suggest digoxigenin-linked biotin linked to the solid support through a reactive oxygen cleavable linker. The Office asserts that the disclosure in the reference at col. 7, lines 10-12, discloses labels, including biotin and digoxigenin, for either end of the peptide, where the labels are linked through a reactive oxygen cleavable linker (referring to the Abstract of Bronstein, first sentence, which mentions 1,2-dioxetanes).

Appellant submits that the patentee, at col. 7, lines 10-12, is merely disclosing alternative ligand binding pairs that may be attached to the peptide to bind or attach the peptide to a solid phase in the heterogeneous assay or to bind to the alkaline phosphatase reagent (see col. 7, ln 13-17). In order for the CSPD reagent of Bronstein to work, the peptide would have to include fluorescein or a fluorescent moiety for the energy transfer to occur. Thus, substituting digoxigenin for fluorescein would render the method of Bronstein inoperable. Even where biotin is employed as the first member of the first ligand binding pair linked to a fluorescein-labeled peptide substrate as disclosed in Bronstein at col. 5, lines 43-48, this reagent and its use in the protease inhibitor assay of the reference does not satisfy the claim language of claim 44. The claim recites digoxigenin-linked biotin linked to the solid support through a reactive oxygen

cleavable linker. In the heterogeneous assay of the reference, cleavage of the peptide, when it occurs, releases a peptide fragment with fluorescein attached but the biotin moiety is left bound to the streptavidin attached to the well. Accordingly, even if for the sake of argument digoxigenin were used in place of fluorescein (a proposition that is unsupported by any teaching in the reference and further it is unclear how Bronstein's method would work without the fluorescein moiety), cleavage would release a peptide fragment with only digoxigenin attached and not biotin. Accordingly, a digoxigenin-linked biotin would not be released from a solid support by a cleavage reaction and, furthermore, a digoxigenin-linked biotin would not be detected.

With regard to the homogeneous assay of Bronstein, (Fig. 14A) for example, as discussed above, in order for the attached CSPD reagent of Bronstein to work, the peptide would have to include fluorescein or a fluorescent moiety for the energy transfer to occur. Thus, substituting digoxigenin for fluorescein would render the method of Bronstein inoperable. Even where biotin is employed as the first member of the first ligand binding pair linked to a fluorescein-labeled peptide substrate as disclosed in Bronstein at col. 7, lines 38-63, this reagent and its use in the protease inhibitor assay of the reference does not satisfy the claim language of claim 44. The claim recites digoxigenin-linked biotin linked to the solid support through a reactive oxygen cleavable linker and further recites (as discussed in more detail below) exciting the sensitizer, which causes the formation of reactive oxygen that cleaves the cleavable linker and releases digoxigenin-linked biotin from the solid support and detecting the released digoxigenin-linked biotin.

In the homogeneous assay of the reference, cleavage of the peptide, when it occurs, releases a peptide fragment with fluorescein attached but the biotin moiety is left bound to the streptavidin attached to the well. Accordingly, even if for the sake of argument digoxigenin were used in place of fluorescein (a proposition that is unsupported by any teaching in the reference and further it is unclear how Bronstein' method would work without the fluorescein moiety), cleavage would release a peptide fragment with only digoxigenin attached and not biotin. Where cleavage of the peptide does not occur, the attached CSPD moiety is cleaved as described and the remaining chemiluminescent moiety attached to one end of the peptide transfers energy to the fluorescein moiety and signal from the fluorescein moiety is detected. The wavelength of the fluorescent emitter is characteristically shifted markedly from that of the dioxetane, allowing

easy discrimination in a homogenous assay. Accordingly, even if for the sake of argument a digoxigenin-linked biotin were released from a solid support by a cleavage reaction the resulting disclosure would still be deficient in not disclosing or suggesting exciting a sensitizer, which causes the formation of reactive oxygen that cleaves the cleavable linker, and in not disclosing or suggesting detecting a digoxigenin-linked biotin.

(3) Limitations of claim 44 that recite exciting the sensitizer where the excitation of the sensitizer causes the formation of reactive oxygen, which cleaves the cleavable linker and releases digoxigenin-linked biotin from the solid support and detecting the released digoxigenin-linked biotin, the amount thereof being related to the amount of analyte in the medium

Neither the homogeneous assay nor the heterogeneous assay of Bronstein includes the above steps. The synthetic peptide reagent for a homogeneous assay (Fig. 14B) is derivatized at both ends of the peptide molecule. Fluorescein, as an energy accepting fluorescent moiety, is at one end of the peptide and a 1,2-dioxetane moiety precursor (Fig. 14A) is at the other end. This reagent may be photooxygenated *in situ* to form the 1,2-dioxetane (Fig. 14B). The 1,2-dioxetane reagent is then employed in a homogeneous assay for the protease inhibitor. See, for example, the discussion at col. 7, lines 38-63, of Bronstein. The peptide is sufficiently short (no more than about 10 amino acid residues) such that the 1,2-dioxetane moiety is in close physical association with the fluorescent label. Upon triggering of the dioxetane, which can be effected by addition of an enzyme, or pH alteration, or application of heat or other triggers, the dioxetane decomposes, emitting energy that excites the fluorescent moiety, which then fluoresces if no cleavage of the peptide has occurred (a positive test for protease inhibition). The triggering of the dioxetane does not involve exciting a sensitizer where the excitation of the sensitizer causes the formation of reactive oxygen, which cleaves the cleavable linker.

The above discussion applies equally to the heterogeneous assay where a separate CSPD reagent is added to the plate after the protease inhibition assay has been carried out to determine the results of the assay. Upon triggering of the dioxetane, which can be effected by addition of an enzyme, or pH alteration, or application of heat or other triggers, the dioxetane decomposes, emitting energy that excites the fluorescent moiety, which then fluoresces if no cleavage of the peptide has occurred (a positive test for protease inhibition). Again, the triggering of the dioxetane does not involve exciting a sensitizer where the excitation of the sensitizer causes the

formation of reactive oxygen, which cleaves the cleavable linker.

The Office has attempted to piece together portions of the above distinct assays of Bronstein in an effort to produce the presently claimed methods. As discussed above, even the pieced together portions are deficient in not teaching or suggesting the elements of the present claims. Furthermore, the Office is required to consider all that a reference discloses; piecemeal reconstruction of the prior art is not allowed. It is not permissible to pick and choose from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art. *In re Wesslan*, 147 USPQ 391, 827 O.G. 348 (1966).

Claim 45 is patentable over the combined teachings of Bronstein and Pease by virtue of its dependency from claim 44, which is patentable over the combined teachings of the references as discussed above. If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988) (M.P.E.P. 2143.03).

The final rejection of claim 46 under 35 U.S.C. 103(a) as being unpatentable over Bronstein in view of Pease

Claim 46 was rejected (and the rejection made final) under paragraph (a) of 35 U.S.C. §103 as being unpatentable over Bronstein in view of Pease. With regard to this rejection, Appellant submits that claim 46 is separately patentable over Bronstein in view of Pease. The rejection of claims 44-45 as unpatentable over Bronstein in view of Pease is discussed in the previous section of the Brief on Appeal. The rejection of claim 44 as unpatentable over Bronstein in view of Pease is discussed in the following section of the Brief on Appeal.

Claim 46 depends from claim 44 and includes the additional limitation that the step of detecting the released digoxigenin-linked biotin is carried out by a detection method employing, as a third specific binding pair member, avidin bound to a member of a signal producing system or anti-digoxigenin antibodies bound to a member of a signal producing system or both. The combined teachings of Bronstein and Pease do not teach this limitation of claim 46. Furthermore, there is nothing on the record indicating where the combination of the teachings of Bronstein and Pease teach or suggest such a limitation. Therefore, claim 46 is separately patentable over

Bronstein and Pease, which in combination fail to disclose or suggest the above limitation as well as the limitations of the base claim, namely, claim 44, as enumerated above.

CONCLUSION AND RELIEF SOUGHT

Appellant has demonstrated above that the specification at page 5, lines 1-14, does contain a written description that enables any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same. There is at least one example in the specification that demonstrates that "release of the substrate with formation of a first binding site may be accompanied by unmasking of at least some of a second binding site,"

Appellant has demonstrated above that the specification at page 9, lines 5-22, which contains the sentence "When peroxide or singlet oxygen is generated, an oxidant cleavable linker is cleaved, releasing multiple products" is not indefinite. The context of the paragraph in which the above sentence appears makes it clear to one of ordinary skill in the art how cleaving a linker results in release of multiple products.

Appellant has demonstrated above that the phrase "water insoluble" is a phrase that is well understood by one of ordinary skill in the art. Accordingly, claims 44-46 are not indefinite under 35 U.S.C. 112, second paragraph.

Appellant has demonstrated above that claims 44-45 are patentable over the combined teachings of Bronstein and Pease. Even if for the sake of argument the teachings of the references were combined, the combination of teachings is deficient in not disclosing or suggesting each and every element of the claims. The combination of teachings fails to disclose or suggest (1) a second specific binding pair member bound to a sensitizer where the sensitizer is capable in its excited state of generating a reactive oxygen species, wherein the proximity of first specific binding pair member with the second specific binding pair member is modulated by the presence of the analyte; (2) digoxigenin-linked biotin linked to the solid support through a reactive oxygen cleavable linker; (3) exciting the sensitizer where the excitation of the sensitizer causes the formation of reactive oxygen, which cleaves the cleavable linker and releases digoxigenin-linked biotin from the solid support and detecting the released digoxigenin-linked biotin, the amount thereof being related to the amount of analyte in the medium. Furthermore, substituting a water insoluble support for the adamantyl moiety of Bronstein would at the very

least cause the heterogeneous and homogeneous assays of Bronstein to function inefficiently and perhaps to be inoperable.

Appellant has demonstrated above that claim 46 is separately patentable over the combined teachings of Bronstein and Pease. Even if for the sake of argument the teachings of the references were combined, the combination of teachings is deficient in not disclosing or suggesting each and every element of the claims. In particular, in addition to the failure to disclose or suggest the elements of claim 44 as enumerated above, the combined teachings do not disclose or suggest the additional limitation of claim 46 that the step of detecting the released digoxigenin-linked biotin is carried out by a detection method employing, as a third specific binding pair member, avidin bound to a member of a signal producing system or anti-digoxigenin antibodies bound to a member of a signal producing system or both.

Accordingly, Appellant respectfully requests that the Board of Patent Appeals and Interferences reverse the following objections and rejections:

(a) the objection to the specification at page 5, lines 1-14,

(b) the objection to the specification at page 9, lines 5-22,

(c) the rejection of claims 44-46 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

(d) the rejection under 35 U.S.C. 103(a) of claims 44-45 on the one hand and of claim 46 on the other hand as being unpatentable over Bronstein in view of Pease.

Respectfully submitted,

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CLAIMS APPENDIX

44. A method for determining the presence or concentration of an analyte in a medium, said method comprising:

providing a reaction mixture comprising in combination:

a medium suspected of containing an analyte;

a first specific binding pair member bound to a water-insoluble solid support;

a second specific binding pair member bound to a sensitizer, said sensitizer capable in its excited state of generating a reactive oxygen species, wherein the proximity of the first specific binding pair member with the second specific binding pair member is modulated by the presence of the analyte; and

digoxigenin-linked biotin linked to the solid support through a reactive oxygen cleavable linker;

incubating the reaction mixture;

exciting the sensitizer, said excitation of the sensitizer causing the formation of reactive oxygen, which cleaves the cleavable linker and releases digoxigenin-linked biotin from the solid support; and

detecting the released digoxigenin-linked biotin, the amount thereof being related to the amount of analyte in said medium.

45. The method of claim 44 wherein:

the proximity of the first and second specific binding pair members to one another results from the binding of the first and second specific binding pair members to the analyte;

the sensitizer is a photosensitizer;

the reactive oxygen species is singlet oxygen; and

the excitation step comprises irradiation of the photosensitizer with light.

46. The method of claim 44 wherein:

the step of detecting the released digoxigenin-linked biotin is carried out by a detection method employing, as a third specific binding pair member, avidin bound to a member of a signal producing system or anti-digoxigenin antibodies bound to a member of a signal producing system or both.

EVIDENCE APPENDIX

No additional evidence is submitted.

RELATED PROCEEDINGS APPENDIX

There are no related proceedings.